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the inclusion of new illustrations, this and excellently reproduced illustrations of the physician regardless of specialty.

NEWMAN (Statens Serum Institut, Copenhagen, 1941, pp. 393.

present knowledge of the genus Salmonella. The material is clearly and concisely presented in excellent bibliography. The subject is covered in a clear and nomenclature, cultural methods of types, serological methods of type determinations, pathogenesis and epidemiology, and a detailed account of the biochemical and given and the characteristics and occurrence (particular attention is given the variational variations) whose recognition is essential to the orientation of a number of strains of coli, indicating that the method of antigenic group.

of the practical problems connected with solved. The etiology and epidemiology of methods of isolation and classification of the infections remains only a matter of organization. To be solved many problems of scientific determination of the complete antigenic partial antigens and the determination of and phase variations; the extension of our members of other genera; further work on the study of Salmonella antigens. the worker undertaking the differentiation:

W. A. NEWMAN DORLAND, A.M., M.D. Leatherette, Ed. 19, revised and enlarged. Plain, \$7.00; thumb-indexed, \$7.50. W.

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Workmen's Compensation and Public Liability. A.C.S., Medical Director, New Jersey Revisor, New Jersey Workmen's Compensation Newark City Hospital, Beth Israel Hospital, Lea and Febiger, Phila.

While, as stated in the preface, every accident litigation raises certain medical questions which only a doctor can answer" not every physician under every circumstance is always in possession of the necessarily comprehensive background to enable a correct answer.

In this book the physician may acquire such a background vicariously. The author has had an ample and comprehensive experience and can speak with authority. An invaluable book to all who may be concerned with the many problems arising from accidental injuries.

DEMONSTRATION OF THE ASSOCIATION OF SPECIFICALLY DIFFERENT ALPHA STREPTOCOCCI WITH VARIOUS DISEASES, AND METHODS FOR THE PREPARATION AND USE OF SPECIFIC ANTISERUMS AND VACCINES IN DIAGNOSIS AND TREATMENT*

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Despite the existence of a great volume of evidence that certain strains of alpha or green-producing streptococci are causative of widely different diseases, much confusion still exists. This is true of both ill-defined²³ and clinically well-defined epidemic¹² and non-epidemic⁴¹ diseases. The reasons for confusion are many, yet quite obvious. Clinicians often do not fully appreciate experimental evidence as regards proof of etiologic importance of inciting agents and bacteriologists all too often work with inadequate methods. Streptococci belonging to this group that have specific properties are usually highly sensitive to oxygen and require special mediums for their isolation and for maintenance of specificity. Conventional mediums now in general use do not suffice. Corroboration of the observations of my colleagues and me has or has not been obtained, depending on whether the principles embodied in our methods^{1,5,8,11,13} have or have not¹⁶ been used. Alpha streptococci having specific properties are associated with diseases at present attributed to virus as now understood. The criterion of death or survival of inoculated animals as evidence of virulence does not suffice in studies on the etiologic importance of this group of organisms. Careful search for lesions, and cultures from lesions if found, in inoculated animals that die as well as in those that survive, are essential to prove that the organisms have specific action and etiologic importance. The principle of dissociation or mutation^{16,30}—which makes difficult their classification by cultural and serologic means and the preparation of really specific vaccines—is not considered sufficiently or is overlooked entirely by different investigators. What has been said regarding causation applies with even greater force to the whole question of passive and active immunization with antiserums and vaccines.

By the use of special methods which have been developed during extended studies on the etiologic importance of these streptococci, not only have we been able to isolate them consistently from areas of infection and from systemic lesions in different diseases, but we often have succeeded in reproducing or simulating, in important respects, the corresponding disease pictures. This has been accomplished in animals by various methods of inoculation of strains freshly isolated in dextrose-brain broth—by intravenous and intracerebral and other methods of inoculation, by feeding and, most important, by inducing chronic foci of infection through the devitalization of the teeth of dogs and inoculation of the respective streptococci into them.³¹ Other workers have had similar results.^{8,11,13}

* Read before the meeting of the American Society of Clinical Pathologists, Cleveland, Ohio, May 30, 1941.

Moreover, it has been shown that the respective streptococci which localize electively in certain tissues or organs produce "endotoxin" and "exotoxin" which cause lesions in the very same tissues as those in which lesions are produced by the corresponding living streptococci. This was demonstrated by the injection separately of the washed organisms killed by heat or formalin and the filtrate of actively growing cultures. These basic observations indicate that general and localized manifestations of disease may be the result of hypersensitivity or allergy specifically related to the streptococci and their products which have specific affinity for certain tissues.

In addition to the consistent isolation of streptococci and the reproduction of the respective lesions in animals, further evidence in favor of their etiologic importance has been obtained by other wholly unrelated methods. By a special staining method²⁷ diplococci, resembling those in dextrose-brain broth cultures of the streptococcus from poliomyelitis, have been found with the light microscope (fig. 1) and in unstained films with the electron microscope, in filtrates of encephalitic and poliomyelitic viruses. The streptococci from different diseases on isolation in dextrose-brain broth were found to have distribution curves of cataphoretic velocity characteristic not only of different and of closely related diseases but, most important of all, of certain streptococcal diseases involving tissues derived from the mesoderm or ectoderm and entoderm, respectively, or from both (fig. 2). The distribution curves of cataphoretic velocity of the streptococci in dextrose-brain broth were similar in the different diseases studied to those of the washed streptococci in distilled water directly from atria of infection. The need for the study of these streptococci on isolation in dextrose-brain broth before specific properties were lost, and the importance of mutation or dissociation were illustrated especially well by these cataphoretic studies. It was found that on exposure of streptococci in this medium to the high frequency field of electric energy for varying time intervals the distribution curves of cataphoretic velocity characteristic of different diseases could be induced in any given strain and that concomitantly changes in localizing power characteristic of the distribution curves of cataphoretic velocity often occurred. As the distribution curve of "neurotropic" streptococci became "arthrotropic" the streptococci lost "neurotropic" virulence and acquired "arthrotropic" virulence, and vice versa.⁴

Horses have been immunized in parallel manner by the repeated intravenous and subcutaneous injection of increasing doses of different strains of streptococci freshly isolated in dextrose-brain broth. The number and interval of injections, the doses of the different strains, and the bleedings were made in parallel manner.* The only variable was the disease from which the different strains were isolated.

The strains used for immunization were isolated either from the systemic lesions of the patient or from respective lesions produced in animals by appropriate inoculations of

* I am indebted to the Research Laboratories of Eli Lilly and Company for their cooperation in immunizing the horses and for making available for study on request the antiserums, the skin test material and the different vaccines prepared by my methods.

streptococci isolated from atria of infection. Pure cultures of the streptococcus then were grown for eighteen hours in large amounts of 0.2 per cent dextrose broth. The organisms were harvested with a continuous-feed centrifuge and immediately suspended in glycerin

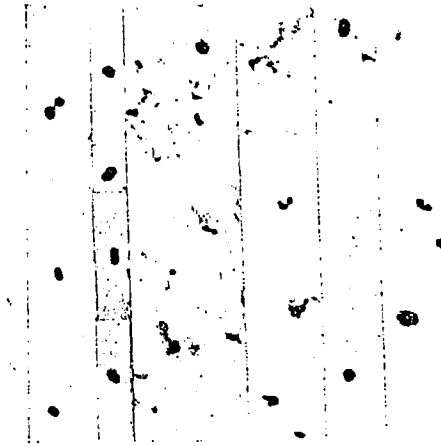


FIG. 1. PHOTOMICROGRAPHS OF DIPLOCOCCI OF VARYING SIZE AND SHAPE, STAINED BY A SPECIAL METHOD, IN A FILTRATE OF AN EMULSION OF THE SPINAL CORD OF A MONKEY THAT SUCCEMDED TO POLIOMYELITIS AFTER INOCULATION OF NATURAL POLIOMYELITIC VIRUS (X 1,000).

Groups (Read up)		Per cent	Electrophoretic time and mobility of streptococci			
Well persons (62,167,2388)		30 20 10				
Diseases af- fecting tissues derived from:	Mesoderm and ectoderm (64,264,4680)	30 20 10				
	Mesoderm (71,169,1708)	30 20 10				
	Ectoderm or entoderm. (163,292,5304)*	30 20 10				
Time in seconds			2.0	3.0	4.0	5.0
Microns per second, volt per centimeter			3.45	2.30	1.72	1.38

FIG. 2. TYPES OF DISTRIBUTION CURVES OF ELECTROPHORETIC TIME AND MOBILITY OF STREPTOCOCCI, ACCORDING TO THE EMBRYOLOGIC ORIGIN OF THE TISSUES AFFECTED. The figures in parenthesis indicate, respectively, the number of strains, cultures and streptococci timed in each group studied.

(two parts) and 25 per cent solution of sodium chloride (one part), the growth from approximately 500 cc. of broth being added to 1 cc. of menstruum. The suspensions then were stored in the refrigerator and appropriate dilutions in solution of sodium chloride were made as needed for injection into horses.

To obtain highly specific antistreptococcal serums it does not suffice to inject streptococci after prolonged cultivation nor as grown on artificial mediums during the long period of immunization, because of their tendency to undergo change.^{19,23} The results obtained in animals, in cataphoretic studies on streptococci and on the diagnostic value of antisera together with a review on elective localization, bibliography and interpretation of the studies on focal infection are available.^{6,34}

THE PRECIPITATION REACTION

For precipitation tests swabbings were made from the nasopharynx high up behind the palate with cotton-wrapped aluminum wire swabs bent to a suitable angle. Care was taken

TABLE 1
PRECIPITATION REACTION BETWEEN SALINE EXTRACTS OF NASOPHARYNGEAL SWABBINGS AND HOMOLOGOUS AND HETEROLOGOUS ANTISTREPTOCOCCIC SERUMS

SOURCE OF EXTRACTS OF NASOPHARYNGEAL SWABBINGS (ANTIGENS)	CASES	EXTRACTS	PERCENTAGE INCIDENCE OF PRECIPITATION BY ANTISERUMS PREPARED WITH STREPTOCOCCI FROM:						NORMAL HORSE SERUM
			Encephalitis	Polio-myelitis	Arthritis	Influenza	Ulcerative colitis	Epilepsy	
Acute and chronic encephalitis.....	11	11	73	27	18	27	27	36	9
Acute epidemic poliomyelitis.....	60	60	42	88	23	12			11
Chronic infectious arthritis.....	7	7	43	14	71	43	29	43	0
Acute influenza.....	70	70	16	14	34	69	20	34	1
Chronic ulcerative colitis and acute epidemic gastroenteritis.....	6	6	0	0	0	67	67	0	0
Epidemic and postoperative persistent hiccup.....	22	26	61	23	35	65	51	73	8
Ether convulsions.....	21	35	74	46	51	43	40	69	11
Miscellaneous infections.....	38	41	24	7	32	41	32	12	5
Well persons and persons having noninfectious diseases remote from epidemics.....	60	68	4	0	3	4	6	0	0

not to touch the tongue with the swab. The adherent material was washed off in 2 cc. of gelatin (0.2 per cent) Loëke solution by thorough shaking and cleared by centrifugation. The serum used as antigen was obtained from the blood of patients drawn before breakfast. The cleared extracts and clear serums, respectively, were superimposed on the respective antisera with as little admixture as possible in small precipitation tubes. Readings were made, after incubation for one and a half hours at 35°C. and after refrigeration over night, under the edge of the shade of a 75 watt electric light in a dark room against a non-reflecting black velvet cloth background. The degree of precipitation was usually slight but only those reactions were recorded as positive in which there was no question of clouding at the interphase.

The precipitation reactions between extracts of nasopharyngeal swabbings of persons ill with various diseases and the homologous and heterologous antistreptococcal serums are summarized in table 1, and those obtained with the

serums from patients and these antistreptococcal serums are summarized in table 2. A high incidence of reactions and a high degree of specificity with the homologous serums were obtained. The serum of horses during the acute stage of encephalomyelitis³⁴ was precipitated as specifically as the serum of persons having encephalitis by the encephalitis antistreptococcal serum prepared with strains from the disease in human subjects. The incidence of reactions with heterologous antisera was greater with extracts of nasopharyngeal swabbings, as should be expected, than with the serums, and in both groups of diseases was highest in those serums prepared with streptococci most nearly resembling the specific strains, such as with the epilepsy serum in the group of patients having epidemic or postoperative persistent hiccup or ether convulsions.³⁶ Proper

TABLE 2
PRECIPITATION REACTION BETWEEN THE SERUM OF PATIENTS AND HOMOLOGOUS AND HETEROLOGOUS ANTISTREPTOCOCCIC SERUMS

SOURCE OF SERUMS (ANTIGENS)	CASES	SERUMS	PERCENTAGE INCIDENCE OF PRECIPITATION BY ANTISERUMS PREPARED WITH STREPTOCOCCI FROM:						NORMAL HORSE SERUM
			Encephalitis	Polio-myelitis	Arthritis	Influenza	Ulcerative colitis	Epilepsy	
Acute and chronic encephalitis.....	95	97	60	20	8	3	5	4	0
Acute epidemic poliomyelitis.....	81	83	31	61	8	0	0	0	0
Chronic infectious arthritis.....	33	33	6	3	55	15	3	0	0
Acute influenza.....	33	33	0	0	0	42	9	3	0
Chronic ulcerative colitis and epidemic gastroenteritis.....	21	21	0	0	5	0	71	0	0
Epidemic and postoperative persistent hiccup.....	24	30	37	0	0	27	10	33	0
Ether convulsions.....	11	12	50	0	0	8	17	25	0
Miscellaneous infections.....	43	43	12	5	14	19	2	7	0
Well persons and persons having noninfectious diseases remote from epidemics.....	108	108	4	2	6	5	2	0	0

dilutions of cleared extracts of the different streptococci preserved in dense suspension in glycerin and salt solution often were precipitated specifically by the homologous antisera.

THE REACTION AFTER CUTANEOUS INJECTION OF ANTIBODY

It was found in previous studies²⁹ that intradermal injection of the whole antistreptococcal serums prepared by intravenous injection exclusively, and in the present study that injection of the euglobulin fraction of the serum of horses immunized by both intravenous and subcutaneous injection, were followed immediately (five to ten minutes) by an erythematous-edematous reaction at the site of injection of the homologous antibody,^{32, 36, 39} but by little or no reaction



at the site of injection of heterologous antibody. This reaction is similar to the reaction first described by Foshay⁷ in tularemia and by Tamura¹⁸ in venereal lymphogranuloma. In our test if the patient's skin or serum contains antigen immunologically related to the antigen or streptococcus with which the reacting antibody is prepared, an immediate transient flare occurs (fig. 3). The flare is taken to indicate the presence of unneutralized antigen in the serum or skin due to an infection by streptococci antigenically identical or related to the streptococcus used to prepare the reacting antibody.

In order to determine more precisely the nature of this reaction, identical numbers of the dead streptococci from poliomyelitis and arthritis were injected intracerebrally into two series of rabbits and *Macacus rhesus* monkeys. The cutaneous tests in rabbits proved unsatisfactory. The monkeys receiving the poliomyelitic streptococci reacted positively to

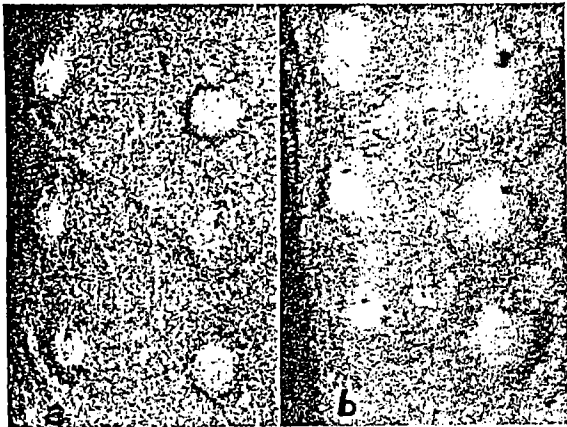


FIG. 3. CUTANEOUS REACTION (a) IN FIVE MINUTES (CONTROL) AND (b) IN THIRTY MINUTES, IN A MONKEY ON THE THIRD DAY AFTER ONSET OF POLIOMYELITIS FOLLOWING INOCULATION OF "NATURAL" POLIOMYELITIC VIRUS

From above down, left row of *a* and *b*: slight reaction in *b* to encephalitis, and no reaction to arthritis and normal horse euglobulins respectively; right row of *a* and *b*: slight reaction in *a* and marked edematous reaction in *b* to three different batches of the poliomyelitic euglobulin.

intra-dermal injection of the poliomyelitic euglobulin but not to the arthritic euglobulin. Those receiving the arthritic streptococci reacted to intra-dermal injection of the arthritic euglobulin but not to the poliomyelitic euglobulin. Precipitation reactions with the serums of both monkeys and rabbits and the respective homologous antisera were positive during the period when the cutaneous reaction was positive. Moreover, the poliomyelitic streptococci remained in the spinal fluid much longer than the arthritic streptococci but did not appear in the knee joint fluid (fig. 4), whereas the arthritic streptococci disappeared promptly from the spinal fluid but appeared, often in easily demonstrable numbers, in the knee joint fluids, as is well illustrated in figure 5. Control punctures of spinal canal and joints before injection proved that spinal fluid and knee joint fluids were sterile and free from bacteria, and all cultures of suspensions injected and of test spinal fluids and joint fluids proved sterile. All organisms, the skin reactivity to homologous euglobulin and the precipitation reaction disappeared in forty-eight to seventy-two hours after injection. This experiment was repeated with a different set of suspensions of the respective streptococci with the same results.

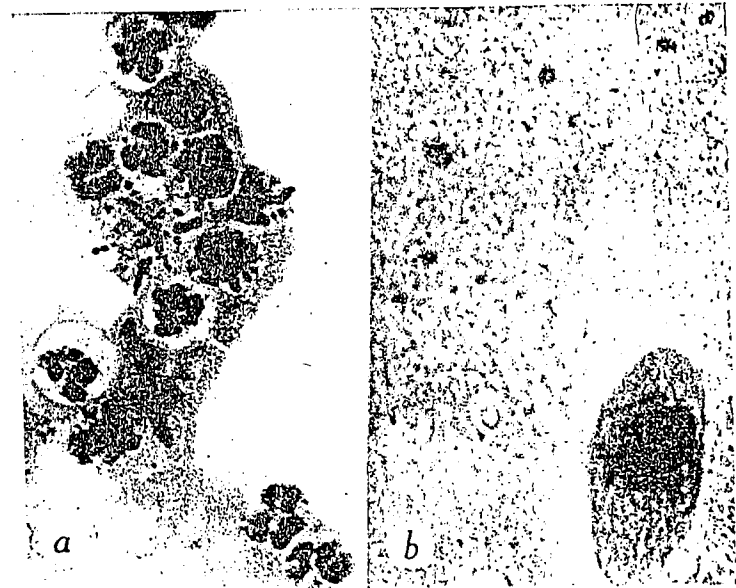


FIG. 4. GRAM STAINED FILMS OF THE SPINAL FLUID (a), AND KNEE JOINT FLUID (b) OF A MONKEY INTO WHICH WERE INJECTED INTRACEREBRALLY THE DEAD (NEUROTROPIC) STREPTOCOCCI FROM POLIOMYELITIS

Note the large number of streptococci in *a* and none in *b* ($\times 1,000$)



FIG. 5. GRAM STAINED FILMS OF THE SPINAL FLUID (a) AND KNEE JOINT FLUID (b) OF A MONKEY INTO WHICH WERE INJECTED INTRACEREBRALLY THE DEAD (ARTHROTROPIC) STREPTOCOCCI FROM ARTHRITIS

Note the absence of streptococci in *a* and the large numbers in *b* ($\times 1,000$)

Striking evidence of specificity of the cutaneous reaction to injection of antibody prepared with streptococci isolated from persons having the corresponding disease was obtained in nearly all of a large number of patients tested. The results are summarized in table 3, in which both the average reaction and the percentage incidence of reactions 5 sq. cm. or more are shown.

Routinely, 0.03 cc. of a 10 per cent solution, in saline containing 0.2 per cent phenol, of the euglobulin fraction (obtained by diluting the serum 1 to 10 in slightly acidulated distilled water) of the different antisera, and of normal horse serum diluted 1 to 10, was injected as superficially as possible into the skin, usually of the forearm. The size of the maximal flare of erythema was outlined with pen and ink and then traced on transparent paper. From such tracing the area of erythema was determined. Maximal reaction usually occurred within five to ten minutes, after which the erythema rapidly disappeared. Slight late (three to four or more days) reaction associated with itching sometimes occurred owing to local sensitization to horse protein.

The cutaneous reactions to the different homologous antibodies were so nearly alike in persons having diseases manifesting similar symptoms and affecting tissues or organs of the same embryologic origin that these are considered in groups for the sake of brevity.

The degree of reaction was highest after injection of the encephalitis euglobulin in the group of patients having encephalitis or spasmodic torticollis. The group having persistent hiccup, epilepsy or other convulsions, in which spasms of muscles suggesting involvement of the brain were the outstanding manifestations, reacted maximally to the encephalitis and epilepsy euglobulins. The persons who had acute poliomyelitis and the group of persons who had chronic poliomyelitis, progressive muscular atrophy, or multiple or amyotrophic lateral sclerosis, in accord with the involvement of brain and cord, reacted maximally to the encephalitic and poliomyelitic euglobulins. The persons who had epidemic poliomyelitis in which only the spinal cord was affected reacted specifically, chiefly to the poliomyelitic euglobulin.

The group that had chronic infectious arthritis, fibrositis, myositis or heart disease reacted maximally and very specifically to the euglobulin prepared with streptococci from arthritis and rheumatic fever. The group representing infections of the respiratory tract—influenza, colds, bronchitis, asthmatic bronchitis and bronchopneumonia—reacted similarly and maximally to the euglobulin from the serum of horses immunized with streptococci from influenza.

The group of persons suffering from gastro-intestinal diseases—ulcer of the stomach, chronic ulcerative colitis and epidemic gastro-enteritis—reacted maximally to the euglobulin prepared with the streptococcus of ulcerative colitis. The twenty persons who had ulcer of the stomach or duodenum included in this group reacted even more strongly to the euglobulin prepared with the streptococcus from ulcer than to the ulcerative colitis euglobulin. The average reaction to the former in clinical cases of ulcer measured 13 sq. cm. and in 77 per cent of the cases there were reactions measuring 5 sq. cm. or more, whereas the reaction to the ulcerative colitis euglobulin approximated that for the gastro-intestinal group. The reactions to the ulcer euglobulin in the groups of persons having encephalitis or arthritis were far less than those of patients having ulcer.

The reactions of well persons to the different euglobulins were uniformly slight, indicating that the beta streptococci normally present in well persons are different from those in persons having symptoms attributable to specific strains. The distribution curves of alpha streptococci from well persons remote from epidemic respiratory infections were like those of specific strains after prolonged

TABLE 3
ERYTHEMATOUS REACTION TO THE INTRADERMAL INJECTION OF THE EUGLOBULIN FRACTION OF HOMOLOGOUS AND HETEROLOGOUS ANTISTREPTOCOCCIC SERUMS

DISEASE GROUPS	CASES	REACTION TO EUGLOBULIN FROM SERUMS OF HORSES IMMUNIZED WITH STREPTOCOCCI FROM:					NORMAL HORSE SERUM	
		Encephalitis	Poliomyelitis	Epilepsy	Arthritis	Influenza		Ulcerative colitis
Encephalitis, spasmodic torticollis.....	86	163*	49	30	75	16	9	73
		7:68	4:47	4:30	5:37	5:43	4:44	1:1
Hiccup, epilepsy, ether convulsions.....	40	53	15	32	29	17	2	31
		6:66	4:33	8:65	2:21	5:29	2:0	1:3
Acute poliomyelitis.....	10	28	18	4	11	12		13
		8:75	10:77	2:0	4:45	2:33		1:17
Chronic poliomyelitis, progressive muscular atrophy, multiple and amyotrophic lateral sclerosis.....	21	34	34	8	17	2		13
		5:41	8:70	4:25	2:6	3:0		1:0
Acute epidemic poliomyelitis.....	63	63	63		63			63
		3:41	8:90		3:21			0:3
Arthritis, fibrositis, myositis, cardiac disease.....	46	53	13	1	43	11	7	31
		4:40	4:31	5:0	10:83	5:54	6:57	1:6
Influenza, colds, bronchial asthma, bronchopneumonia.	65	41	13		27	62	13	10
		2:17	2:7		2:18	10:81	2:46	1:0
Ulcer of stomach, chronic ulcerative colitis, epidemic gastro-enteritis.....	41	27	6		27	17	29	31
		5:41	2:0		4:44	6:29	9:72	1:0
Well persons remote from epidemics.....	84	91	93		81	77	70	84
		1:13	1:8		2:13	1:17	1:7	1:0

* The figures above the line in each instance indicate the number of tests; the two sets of figures below the line indicate, respectively, the average reaction in square centimeters and the percentage of reaction measuring 5 sq. cm. or more. Fractions are given to the nearest whole number.

cultivation on artificial mediums.²⁵ The former streptococci, as the latter, also lacked specific virulence.^{17, 34} The reactions to injection of normal horse serum diluted 1 to 10, as recorded in table 3, and to a 10 per cent solution of normal horse euglobulin in special instances were similar and uniformly slight, aside from those in a few persons sensitive to horse serum.

In extended studies on the diagnostic value of the cutaneous test, especially in connection with poliomyelitis and influenza, a striking difference in the reactivity of the skin to heterologous euglobulins was noted in certain groups of patients and well persons. This was found to be dependent, in part, on density of population in which persons were residing, whether in large cities or in rural communities. The reactions, as usual, to homologous euglobulins were maximal in both groups but reactions to heterologous euglobulins were greater in the urban than in the rural groups. This has been observed repeatedly and suggests subclinical but immunizing infection by antigenically different streptococci. This observation is in accord with the generally accepted view that city dwellers have a higher level of resistance when exposed to epidemic respiratory and other infections than persons residing in the country. A relatively higher incidence of reactors to the euglobulins characteristic of epidemics was found among well persons, whether exposed directly or not, within epidemic zones than among persons remote from epidemics, irrespective of density of population. This is taken to indicate the wide prevalence of the causative streptococcus and perhaps the corresponding virus and of subclinical immunizing infections, and affords—it would seem—explanation as to why epidemics, especially in rural communities, run their course usually within eight weeks, and why epidemics in which enduring immunity to the disease in question is conferred do not recur except at long intervals. Specific antibody content of convalescent serum was usually not sufficiently high to yield clear-cut cutaneous reactions. A few serums from persons convalescing from acute epidemic poliomyelitis²⁹ and from recurring encephalomeningoradiculitis with fibromyositis³³ gave suggestive results.

METHODS AND RESULTS OF AGGLUTINATION TESTS

The difficulties encountered in agglutination experiments with streptococci in general and with the green-producing group in particular, when made by conventional methods, have been largely overcome by the use of methods which we have developed. Green-producing streptococci which are causative of certain diseases have been found highly sensitive to oxygen. Ordinary mediums often do not suffice for their isolation, a fact not sufficiently considered in studies on etiology and in the preparation of vaccines. Hence, the primary isolations are made by us in the highly favorable medium, dextrose-brain broth,²² which affords a gradient of oxygen tension, a low oxidation-reduction potential, and other conditions highly favorable for growth of sensitive streptococci. The dextrose-brain broth consists of 0.2 per cent ordinary dextrose broth adjusted to pH 7.2, and the soft dextrose-brain agar also used likewise consists of dextrose broth containing 0.2 per cent agar, to both of which approximately one part of pieces of freshly obtained calf brain to six or seven parts of broth in tall (10 cm.) columns is added before autoclaving.

Pure cultures of freshly isolated strains were obtained not by plating on blood agar but by inoculation of animals or by making serial dilution cultures alternately in dextrose-brain broth and dextrose-brain agar at steps of 10 to 10,000, depending on the character of the

inoculum.³¹ The streptococci to be used for agglutination experiments and for the preparation of prophylactic and therapeutic vaccines were grown usually for eight to eighteen hours in dextrose-brain broth. The supernatant culture was transferred to test tubes or bottles and centrifuged. The supernatant cleared broth was decanted and the streptococci were suspended routinely in the menstruum of glycerin-salt solution so that the suspensions contained approximately 100 times as many streptococci per cubic centimeter as the dextrose-brain broth culture. After thorough mixing the suspensions were placed in the refrigerator.

The dense suspensions in glycerin-salt solution and the respective antisera were diluted in physiologic solution of sodium chloride containing 0.2 per cent phenol for the agglutination tests. The density of the suspensions was adjusted to approximately twice that of a dextrose-brain broth culture. The hyperimmune sera were diluted 1 to 5, 1 to 25, 1 to 125 and 1 to 625, and the sera of patients 1 to 10, 1 to 20, 1 to 40 and 1 to 80. Equal parts of the respective suspensions and serum dilutions were mixed and the setup was placed at 49 to 50°C. for eighteen hours, when readings were made, instead of after being kept at 37°C. for one or two hours and then in the refrigerator over night, as is usually done. The degree of agglutination in the different dilutions was recorded according to the arbitrary scale of 0 to 4. In summarizing results agglutination was considered as specific in the serum that showed greater agglutination than occurred in any of the other antisera used.

The results of agglutination tests made in this way with homologous and heterologous whole antistreptococcal serum or globulin concentrates are summarized in table 4. The results with strains from closely related diseases were so similar as to permit placing them in representative groups for brevity. The results in each of the different groups were highly specific, the incidence of agglutination being far higher in the homologous than in the heterologous antisera. Although occasionally, as table 4 indicates, several tests were made on different cultures isolated from the same patient, these different cultures were as variable in their reactions as the total population of cultures isolated from the different patients, and the total results are not affected by this procedure. This was true also of the distribution and results of the several extracts and sera tested from the same persons in several of the groups summarized in tables 1 and 2. Therefore, the results are given in percentage of cultures, extracts or sera tested rather than percentages of cases.

In the agglutination tests, as was noted in the precipitation and cutaneous tests, the incidence of marked specific action of the epilepsy antiserum was limited to the strains of streptococci isolated in the cases of persistent hiccup and other convulsions. In the case of streptococci isolated in studies of encephalitis, the high point of agglutination was by the antiserum prepared with streptococci isolated in studies of encephalitis in human beings. This serum also agglutinated specifically the strains of streptococci isolated in studies of equine encephalomyelitis. In the group of cases of acute poliomyelitis and progressive muscular atrophy maximal agglutination occurred in the poliomyelitis antistreptococcal serum. In the group of cases of multiple or amyotrophic lateral sclerosis in which both spinal cord and brain are affected, agglutination was high in both the poliomyelitis and the encephalitis antistreptococcal sera. The incidence of specific agglutination of the strains isolated in arthritis was by far the highest in the arthritis antistreptococcal serum; in those from chronic ulcerative colitis

and epidemic gastro-enteritis by the antiserum prepared with the diplostreptococcus of ulcerative colitis, and in the group of strains from persons having colds, influenza, asthma or bronchitis by the antiserum prepared with the streptococcus from influenza.

The incidence of specific agglutination of streptococci isolated in twenty-three cases of myasthenia gravis, not recorded in table 4, was very high (74 per cent) in the antisera prepared with the streptococcus isolated in studies of myasthenia gravis,^{4, 37, 38} and next highest in the antiserum prepared with the streptococcus isolated in studies of arthritis. Absorption of the different antisera

TABLE 4

AGGLUTINATION OF STREPTOCOCCI, AS ISOLATED IN STUDIES OF VARIOUS DISEASES, BY HOMOLOGOUS AND HETEROLOGOUS ANTISTREPTOCOCCIC SERUMS

DISEASE GROUPS	CASES	CULTURES	PERCENTAGE INCIDENCE OF SPECIFIC AGGLUTINATION BY ANTISERUMS PREPARED WITH STREPTOCOCCI FROM:						NO AGGLUTINATION, OR NON-SPECIFIC AGGLUTINATION
			Encephalitis	Poliomyelitis	Arthritis	Influenza	Ulcerative colitis	Epilepsy	
Acute and chronic encephalitis.....	96	144	75	9	10	0	0	3	3
Persistent hiccup and ether convulsions.....	40	125	34	4	1	1	0	49	11
Amyotrophic lateral and multiple sclerosis.....	9	9	44	56	0	0	0	0	0
Progressive muscular atrophy and chronic poliomyelitis.....	16	16	19	75	0	0	0	6	0
Acute epidemic poliomyelitis.....	146	229	10	67	9	3	0	0	11
Chronic infectious arthritis and rheumatic arthritis.....	42	47	7	0	85	4	2		2
Colds, influenza, asthma and bronchitis.....	86	144	7	3	4	69	2	14	0
Chronic ulcerative colitis and epidemic gastro-enteritis.....	36	51	8	4	4	10	55	8	12
Miscellaneous diseases....	49	49	8	4	22	14	0	16	35

with homologous streptococci removed their specific precipitating and agglutinating power. Streptococci from persons having miscellaneous diseases were not markedly nor differentially agglutinated by any of the antistreptococcus serums used.

The agglutinin content of the serum of persons having certain low grade illness is often too small to permit of clear-cut demonstration, even by our methods. However, well marked evidence was obtained in some instances of an increase of specific agglutinins during convalescence or continued infection. The results

of experiments with a large number of strains and serums in acute and chronic encephalitis, poliomyelitis and arthritis are summarized in table 5. Well marked evidence of specific agglutinins in each of these diseases was found. Similarly, an increase in agglutinins specific for the strains of streptococci isolated consistently in the St. Louis epidemic of encephalitis occurred during convalescence. Thus, the incidence of agglutination, over the strains of streptococci isolated, in 170 tests made with eleven serums obtained one to seven days after illness began was 62 per cent; at eight to fourteen days with six serums it was 81 per cent of 132 tests, and at fifteen to twenty-one days with forty-five serums agglutination of the encephalitis streptococcus occurred in 84 per cent of 478 tests. In contrast, fifteen control serums used under identical conditions gave an incidence of agglutination of only 24 per cent of 228 tests. There was a similar, although a less marked, increase in agglutinating titer of these convalescent serums over the streptococci isolated in studies of poliomyelitis, but no increase of the agglutinating power over strains of streptococci isolated in arthritis and cholecystitis used as controls. Easily demonstrable increased agglutinating power over streptococci isolated from the blood during the acute stage of equine encephalomyelitis and from the brain after death developed in the serum of horses during convalescence from the disease.

RESULTS FROM THE USE OF ANTISTREPTOCOCCIC SERUMS AND STREPTOCOCCIC VACCINES

Results from the use of the antisera prepared in the way indicated in the treatment of epidemic poliomyelitis^{35, 43} and of encephalitis^{10, 18} have been reported. Concomitantly with subjective improvement noted after approximately 0.5 cc. per kilogram of body weight of the globulin concentrate of the antiserum (one part euglobulin containing chiefly bacterial antibody and two parts pseudoglobulin containing chiefly antitoxic antibody) had been given, there occurred a prompt (two to four hours) diminution in the reactivity of the skin to reinjection of the respective euglobulins, but the reactivity reappeared to a lesser degree in eighteen to twenty-four hours. Two intramuscular injections per day for several days usually sufficed to bring the temperature and cutaneous reactivity to normal with concomitant alleviation of symptoms. Similar, as yet unpublished, results have been obtained with the encephalitis and epilepsy antistreptococcic serums in a series of cases of persistent epidemic or postoperative hiccup, in which the patients usually reacted strongly to both the encephalitis and epilepsy euglobulins. Similar striking results have been obtained in acute influenza with the influenza antistreptococcic serum. Improvement, sometimes striking, or staying of the process occurred in cases of chronic encephalitis,¹² chronic poliomyelitis, chronic ulcerative colitis³ or chronic infectious arthritis, by intramuscular injection twice weekly of subsensitizing doses of the respective concentrated antisera. The schedule of dosage in these cases consisted of the intramuscular injection of 0.1 cc. and increasing by 0.1 cc. up to 2 cc., provided untoward reactions did not occur and favorable ef-

fects were noted. Detailed reports of the further use of antistreptococcic serums and vaccines are beyond the scope of this paper.

Results, often striking, from the use of the vaccines prepared from the suspensions of streptococci in glycerin-salt solution in prevention and treatment of experimental infections in animals and in epidemic and other diseases in human beings and horses have been set forth in a series of reports.^{2, 12, 14, 25, 40, 45} Vaccines prepared from streptococci that have been preserved in glycerin-salt solution are much less toxic and more promptly antigenic²⁵ than corresponding vaccines prepared with streptococci directly from cultures. By virtue of these properties they are better suited not only for prophylaxis but especially for treatment than when prepared directly from cultures. No untoward reactions⁹

TABLE 5

AGGLUTINATION OF STREPTOCOCCI FROM POLIOMYELITIS, ENCEPHALITIS AND ARTHRITIS BY THE RESPECTIVE CONVALESCENT SERUMS

CONVALESCENT SERUMS FROM PERSONS HAVING	STREPTOCOCCI FROM PERSONS HAVING					
	Poliomyelitis		Encephalitis		Arthritis	
	Strains	Per cent showing agglutination at dilutions of 1 to 80	Strains	Per cent showing agglutination at dilutions of 1 to 80	Strains	Per cent showing agglutination at dilutions of 1 to 80
Poliomyelitis.....	195 (104)*	56	51 (41)	26	48 (41)	25
Encephalitis.....	46 (25)	19	82 (42)	54	55 (42)	29
Arthritis.....	24 (16)	4	60 (45)	12	91 (45)	69
Controls: well persons.....	55 (26)	9	39 (34)	18	39 (34)	21

* The figures in parentheses indicate the number of serums used. Agglutination tests were not made with all of the different serums and all of the strains, but the number made in each of the groups was more than 1,000 and therefore the percentages given have undoubted statistical significance. Percentages are given to the nearest whole number.

have occurred after the injection of many thousands of properly gauged doses of the streptococcic vaccines prepared in this way.

COMMENT AND CONCLUSIONS

Studies on alpha or green-producing streptococci associated with certain epidemic and non-epidemic diseases are reported. Special methods were found necessary for the consistent isolation of strains that had specific properties, for the demonstration of specific virulence, for the maintenance of specific antigenicity and for the preparation of highly specific antisera and vaccines. Studies to be reported elsewhere indicate that the respective specificities, al-

though clear-cut on isolation in each of the diseases studied, are related to environmental, seasonal and other influences.

Analysis of the data in tables 1, 2, 4 and 5 on the differences between the various serums from specifically immunized horses and normal horse serum, and the differences between the serums obtained or cultures isolated from persons suffering or convalescent from specific infections and similar serums or cultures from well persons, shows that these differences were statistically significant, except in a few cases in which the numbers were too small to make comparison possible. The striking parallelism of results obtained by the widely different methods used—inoculation of animals, electrophoresis and the precipitation, cutaneous, agglutination and agglutinin and precipitin absorption reactions—indicates especially their etiologic importance. The closely parallel results obtained from the use of the cutaneous and agglutination tests in the arthritic-rheumatic group of patients are in strict accord with the report by Baggenstoss and Rosenberg¹ of the finding of cardiac lesions identical with those of rheumatic fever in fourteen (56 per cent) of twenty-five cases of chronic infectious arthritis studied.

The mildness or even absence of symptoms suggestive of infection, the small number or apparent absence of organisms in systemic lesions, and the extreme sensitivity to specific vaccines, noted especially in chronic cases, suggest that a local or generalized allergy specifically related to the respective streptococci and to their toxic products is often responsible for the symptoms and progressive course in some of the diseases studied.

It is, of course, to be understood that the evidence, while quite conclusive in some diseases studied, is only suggestive in others. For example, the evidence indicating the possible importance of streptococci in the causation of epilepsy has been obtained in only a relatively small number of cases. Cultures of the blood at the time of seizure have yielded the streptococcus repeatedly. Intracerebral inoculation into animals of suitable doses of the streptococcus freshly isolated from blood and atria of infection produced as the outstanding manifestation localized and generalized spasms of muscles and sometimes recurring generalized convulsive seizures.²⁶ The cutaneous reaction was usually maximal with the homologous euglobulin and the precipitation reaction was consistently positive between the whole homologous antiserum and the serum of patients at the time of seizures. The streptococcus was agglutinated specifically by the homologous antiserum and the antibodies were removed specifically from the antisera in absorption experiments with the streptococcus. Vaccines prepared with this streptococcus seemingly have produced cure in a number of cases of early epilepsy.

The favorable results obtained in studies on the etiology and treatment by antistreptococcic serums and vaccines of chronic poliomyelitis, progressive muscular atrophy, multiple and amyotrophic lateral sclerosis, as well as of thrombophlebitis and pulmonary embolism^{20, 21} (not included in the tables), also are considered only as highly suggestive.

The nature of the causal relation of alpha streptococci having specific properties

o the virus diseases studied and their viruses, as now understood, whether synergistic, dissociative, mutational or phasal, is not entirely clear. Our studies of encephalitis of the fox²⁴ and horse,⁴⁴ of poliomyelitis in man and monkey,²⁸ and of epidemic influenza in man indicate: (1) that the relation is probably chiefly phasal and that these diseases are primarily specific streptococcal infections; (2) that during the course of such infections a virus phase of the streptococcus develops; (3) that the respective streptococci and corresponding viruses are antigenically related, and (4) that with the respective streptococci it is possible to prepare preventive and curative vaccines and antiserums for these as for the other diseases studied.

The methods we have developed are relatively simple, and their general adoption, or the development of better ones in harmony with the underlying principles embodied, would result, it is believed, in a better understanding of the nature and treatment of the puzzling group of diseases associated with or caused by highly specific strains of alpha or green-producing streptococci.

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